Early Developmental Ethanol Exposure Disrupts Hippocampal-Dependent Learning via Deregulated NMDA Receptor Function in a Rodent Model of Fetal Alcohol Syndrome

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Overview

Consuming alcohol during pregnancy may lead to a range of lifelong disorders in the resulting offspring known as fetal alcohol spectrum disorders (FASD). **Fetal alcohol syndrome (FAS)** lies at the severe end of the spectrum. In our FAS rat model, neonate pups are intragastrically intubated with alcohol in a milk formula across postnatal days (PD) 4-9, a period commensurate to the third trimester in human pregnancy.

At ~PD70, rats were submitted to a behavioral task called the **context pre-exposure facilitation effect (CPFE)**, which consists of three phases. First, rats are exposed to a novel context. During exploration, the contexts' features are bound into a single representation via NMDA receptor (NMDAR)-dependent plasticity (Rudy and O'Reilly, 2001). Second, rats are returned to the same context and presented with an immediate (~8 sec) footshock and removed. In the time prior to the shock, pattern completion within the hippocampus allows retrieval of the entire representation (Kesner, 2007), which is then associated with the aversive footshock. In phase three, rats are again placed in the context and conditioned fear (freezing) is measured. Successful retrieval of the contextual representation and its association with the shock results in elevated contextual freezing at test.

Two different time intervals (2 or 24 h) are used between the pre-exposure and immediate shock in order to investigate the stage of learning (encoding, consolidation, or retrieval) at which FAS rats are impaired. The 2 h interval allows for reliance on a short-term memory representation of the context and does not required consolidation. Data indicate the FAS rats are impaired when trained with the 24 h, but not 2 h, exposure-to-shock interval. Our working hypothesis is that hippocampal NMDARs in the FAS rats operate non-optimally, limiting the consolidation or storage of the contextual memory. In support, preliminary data indicate administration of an NMDAR partial agonist, **D-cycloserine (DCS)**, prior to initial context exposure facilitates learning in FAS rats, when phase 1 and 2 are separated by 24 h, as evidenced by enhanced freezing at test.

FAS Rodent Model

Long-Evans rat pups pseudo-randomly assigned to one of three treatment groups:

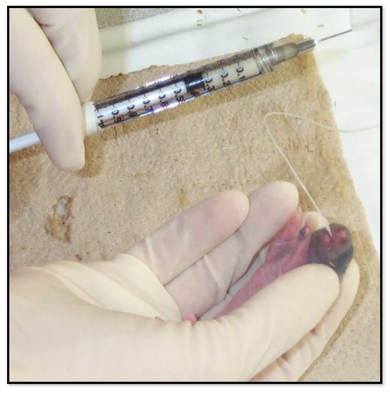
UC: Unhandled control
pups removed but not intubated

SI: Sham-intubated
pups intubated but no alcohol given

5E: Ethanol exposure 11.33% (5g/kg/day)
pups intubated across postnatal days (PD) 4-9

Footshock: 1.0 mA, 2.0 sec
D-cycloserine: 15 mg/kg (IP)

Peak BAC: 349.1 ± 12.3 mg/dl

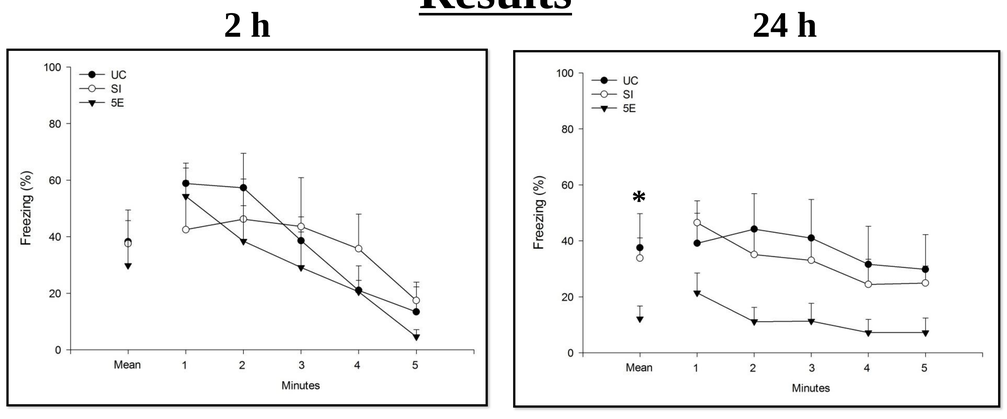


Training Paradigm

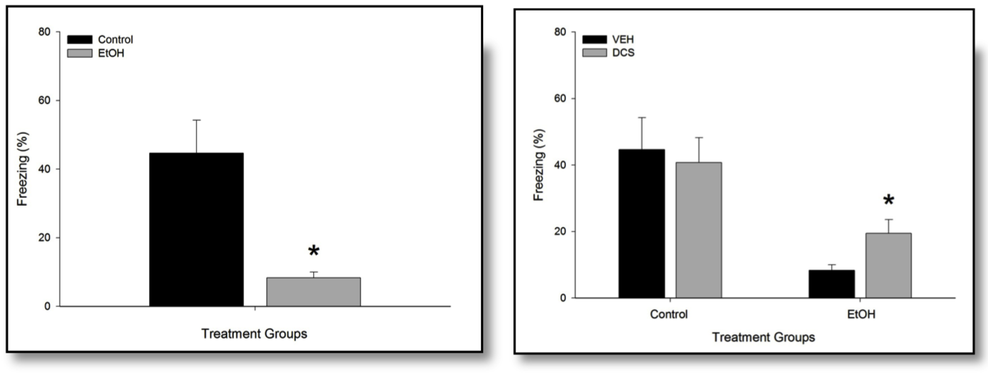
CPFE: Three Phases
Phase 1: Context pre-exposure (5 min)
Phase 2: Immediate footshock
Phase 3: Fear test (5 min)

Phase 1 and 2 separated by 2 or 24 h

Results



Fear testing in rats pre-exposed to the context and then submitted to an immediate footshock 2 or 24 h later. Results indicate only with the long context exposure-to-shock interval (24 h) are FAS rats significantly impaired relative to control animals.



Fear testing in control and FAS rats trained with a 24 h context exposure-to-shock interval (left). Rats injected with vehicle or DCS approximately 30 min prior to context exposure. The NMDAR partial agonist significantly enhanced freezing in FAS rats (right).

Results

- FAS rats were impaired in the 24 h CPFE task relative to controls.
- FAS rats show comparable rates of freezing in the 2 h CPFE relative to control, indicating they can successfully encode a memory representation of the context.
- Results suggest that the 24 h ethanol-induced CPFE impairment is due to a consolidation deficit in FAS rats, possibly the result of NMDAR hypofunction.
- DCS administration prior to context pre-exposure significantly enhanced freezing rates at test in FAS rats, supporting our hypothesis that NMDAR function is non-optimal in FAS rats.

Future Directions

- Utilize Western blotting to assess NMDAR-dependent plasticity via expression of the downstream biomarker activity-regulated cytoskeleton-associated (Arc; Arg3.1).
- Quantify the NMDARs constituent subunits within the hippocampus. The subunits are known to be expressed at different rates across development, a process that ethanol is proposed to deregulate.
- Clarify the temporal window of vulnerability for early ethanol exposure. Currently, rats are intubated across PD4-9. Learning will be assessed in rats with ethanol exposure restricted to PD 4-6 or 7-9.

References

O'Reilly, Randall C.; Rudy, Jerry W. Conjunctive representations in learning and memory: Principles of cortical and hippocampal function. Psychological Review, 108(2), Apr 2001.

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